

3

5

10

15

20

25

30

35

5. The method according to claim 4, wherein the bone marrow is cultured with rGM-CSF at a concentration of about 500-1000 U/ml.

6. The method according to claim 5 wherein Ia-negative marrow nonlymphocytes are cultured at a concentration of about 5×10^5 cells/cm².

7. The method according to claim 6, wherein the anti-Ia antigen antibodies and anti-T cell, B cell and monocyte antibodies are selected from the group consisting of GK 1.5 anti-CD4, Ho 2,2 anti-CD8, B21-2 anti-Ia, and RA3-3A1/6.1 anti-B220/CD45R.

8. The method according to claim 3 wherein the cell aggregates of step (e) are serially subcultured one to five times.

9. The method according to claim 8 wherein the cell aggregates are serially subcultured two to three times.

10. The method according to claim 9 wherein the cell aggregates are serially subcultured two times.

11. The method according to claim 3 wherein the nonadherent cells and cell clusters of step (c) are subcultured after from about 0.3 to 1 day and the cell aggregates are serially subcultured every 3 to 30 days.

12. The method according to claim 11 wherein the cell aggregates are serially subcultured every 10 to 20 days.

13. The method according to claim 12 wherein the cell aggregates are serially subcultured every 20 days.

14. The method according to claim 3 wherein the tissue source is blood or bone marrow, the nonadherent cells and cell clusters of step (c) are subcultured after about 0.3 to 1 day, the cell aggregates are serially subcultured one to five times every 3 to 30 days.

15. The method according to claim 14 wherein the nonadherent cells and cell clusters of step (c) are subcultured after about one half day and the cell aggregates are twice serially subcultured after 20 days.

16. The method according to claim 3 wherein the culture medium is selected from the group consisting of RPMI 1640, DMEM, and α -MEM and wherein the culture medium is supplemented with serum.

17. The method according to claim 16 wherein fetal calf serum is present in the culture medium in an amount of about 1 to 15%.

18. The method according to claim 17 wherein the fetal calf serum is present in the culture medium in an amount of about 10%.

19. The method according to claim 14 wherein the tissue source is blood and the concentration of GM-CSF in the medium is about 30-100 U/ml.

20. The method according to claim 14 wherein the tissue source is bone marrow and the concentration of GM-CSF in the medium is about 500-1000 U/ml.

21. A method of producing a population of mature dendritic cells from proliferating cell cultures comprising:

a) providing a tissue source comprising dendritic cell precursors;

b) treating the tissue source from (a) to increase the proportion of dendritic cell precursors to obtain a population of cells suitable for culture in vitro;

c) culturing the tissue source on a substrate in a culture medium comprising GM-CSF to obtain nonadherent cells and cell clusters;

d) subculturing the nonadherent cells and cell clusters to produce cell aggregates comprising proliferating dendritic cell precursors;

e) serially subculturing the cell aggregates one or more times to enrich the proportion of dendritic cell precursors; and

f) continuing to culture the dendritic cell precursors for a period of time sufficient to allow them to mature into mature dendritic cells.

22. The method according to claim 21 wherein the tissue source is blood or bone marrow and GM-CSF is present in the medium at a concentration of about 1-1000 U/ml.

23. The method according to claim 21, further comprising that when the tissue source is bone marrow the pretreatment step comprises killing cells expressing antigens which are not expressed on dendritic precursor cells by contacting the bone marrow with antibodies specific for antigens not present on dendritic precursor cells in a medium comprising complement.

24. The method according to claim 23, wherein the tissue source is bone marrow and the antibodies are directed against at least one antigen selected from the group consisting of Ia antigen, antigens present on T cells, and antigens present on mature dendritic cells.

25. The method according to claim 24, wherein the bone marrow is cultured with rGM-CSF at a concentration of about 500-1000 U/ml.

26. The method according to claim 25 wherein Ia-negative marrow nonlymphocytes are cultured at or concentration of about 5×10^5 cells/cm².

27. The method according to claim 25 wherein the anti-Ia antigen antibodies and anti-T cell, B cell and monocyte antibodies are selected from the group consisting of GK 1.5 anti-CD4, Ho 2.2 anti-CD8, B21-2 anti-Ia, and RA3-3A1/6.1 anti-B220/CD45R.

28. The method according to claim 21 wherein the cell aggregates of step (e) are serially subcultured one to five times.

29. The method according to claim 28 wherein the cell aggregates are serially subcultured two to three times.

30. The method according to claim 29 wherein the cell aggregates are serially subcultured two times.

31. The method according to claim 21 wherein the nonadherent cells and cell clusters of step (c) are subcultured after from about 0.3 to 1 day and the cell aggregates are serially subcultured every 3 to 30 days.

32. The method according to claim 31 wherein the cell aggregates are serially subcultured every 10 to 20 days.

33. The method according to claim 32 wherein the cell aggregates are serially subcultured every 20 days.

34. The method according to claim 31 wherein the cell aggregates are serially subcultured one to five times.

35. The method according to claim 29 wherein the nonadherent cells and cell clusters of step (c) are subcultured after about one half day and the cell aggregates are twice serially subcultured after 20 days.

36. The method according to claim 21 wherein the culture medium is selected from the group consisting of RPMI 1640, DMEM, and α -MEM and wherein the culture medium is supplemented with serum.

37. The method according to claim 36 wherein fetal calf serum is present in the culture medium in an amount of about 1 to 15%.

38. The method according to claim 37 wherein the fetal calf serum is present in the culture medium in an amount of about 10%.

39. The method according to claim 21 wherein the tissue source is blood and wherein GM-CSF is present in the medium at a concentration of about 30-100 U/ml.

40. The method according to claim 21 where the tissue source is bone marrow and wherein the CM-CSF is present in the medium at a concentration of about 500-1000 U/ml.

41. A method for providing an antigen to a host comprising exposing an antigen to a culture of dendritic cells obtained according to the method of any one of claims 21, 39 or 40 to produce antigen-activated dendritic cells followed by inoculating the host with the antigen-activated dendritic cells.

42. The method according to claim 41 wherein the host is human.

43. A composition comprising dendritic cells prepared according to the method of any one of claims 21, 39 or 40.

5 44. A composition comprising antigen-activated dendritic cells wherein dendritic cells prepared according to claim 21 are pulsed with an antigen and wherein the dendritic cells process the antigen to produce a modified antigen which is expressed by the dendritic cells.

10 45. A composition comprising a dendritic cell modified antigen wherein a substance to be modified is exposed to a culture of dendritic cells prepared according to any one of claims 21, 39 or 40 and whereby the substance is modified by the dendritic cells to produce the modified antigen.

15 46. A method of immunizing against disease in humans or animals comprising, administering a vaccine comprising the composition of claim 44.

20 47. A vaccine comprising the composition of claim 44.

25 48. A method of immunizing against disease in humans or animals comprising, administering a vaccine comprising the composition of claim 45.

49. A vaccine comprising the composition of claim 45.

30 50. A method of treating autoimmune disease comprising administering to a person in need of treatment a therapeutically effective amount of the composition of claim 44 and wherein the antigen to be modified is a self-protein.

35

3

5

10

15

20

20

25

30

30

60. The method according to claim 14 wherein the tissue source is human blood and GM-CSF is present in the medium at a concentration of about 400 to 800 U/ml.

5 61. The method according to claim 60 wherein at least one factor selected from the group consisting of TNF- α , G-CSF, IL-1 and IL-3 is present together with GM-CSF in the culture medium.

62. The method according to claim 22 wherein the tissue source is human blood and GM-CSF is present in the medium at a concentration of about 400 to 800 U/ml.

63. The method according to claim 62 wherein at least one factor selected from the group consisting of TNF- α , G-CSF, IL-1 and IL-3 is present together with GM-CSF in the culture medium.

64. The dendritic cell precursors according to claim 56 wherein the cells are obtained from human blood and are cultured in the presence GM-CSF at a concentration of about 400 to 800 U/ml.

25 65. A method of preparing an antigen fragment from an antigen comprising contacting the antigen with cells selected from the group consisting of dendritic cells and dendritic cell precursors, incubating the cells with the antigen for sufficient time to allow the cells to process the antigen into the fragments and present the antigen fragment on the cell surface.

30

66. The method according to claim 65 wherein the dendritic cells or dendritic cell precursors are derived from blood or bone marrow.

3

5

10

15

20

25

30

35

78. The antigen fragment according to claim 75 wherein the antigen is a gene product expressed by a viral vector phagocytosed by the dendritic cell precursors.

10

15

20

25

Add
B'

Good EM

13
30

107

[Handwritten signature]